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14. ABSTRACT: In this project, we propose to develop a new drug delivery vehicle based on dendrimer nanotechnology for personalized medicine. This new class of nanoplatforms contains imaging probe and molecular medicine with a cancer-specific targeting capability which is able to target cancer cells, monitor drug delivery and tumor response to achieve a "see and treat" strategy as a new concept of molecular medicine. Specifically, One Partner PI's lab will make dendrimers bearing functional handles to conjugate with chelating agents provided by the Initiating PI's lab for PET imaging and therapeutic peptides provided by another Partner PI's lab for the treatment of aggressive prostate cancer. In the 1st year, the Initiating PI's lab has designed and synthesized the proposed bifunctional chelator scaffold system, CB-TE2A(^{t}Bu)$_2$-N$_3$ for the further construction of dendrimer-based theranostic agents for aggressive prostate cancer. In the meanwhile, the design concept has been extended to synthesize a bivalent imaging probe for quantitative PET imaging of PSMA expression in prostate cancer.

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INTRODUCTION

This project combines the recent advances in prostate cancer (PCa) research from three different labs integrated with a strong interest and dedication to develop a new molecular medicine approach towards the eventual cure of PCa. Like other cancer types, the current available therapeutic regimens for metastatic PCa are not PCa specific. With respect to PCa cells harboring various genetic alterations, the development of small molecular agents targeting these genetic defects to achieve a better therapeutic efficacy is foreseeable. In this project, we propose to develop a new drug delivery vehicle based on dendrimer nanotechnology for personalized medicine. This new class of nanoplatforms contains imaging probe and molecular medicine with a cancer-specific targeting capability which is able to target cancer cells, monitor drug delivery and tumor response to achieve a "see and treat" strategy as a new concept of molecular medicine. This platform system will be flexible to adopt any new cell targeting molecule or any therapeutic agents. Specifically, Dr. Simanek's lab will make dendrimers bearing functional handles to conjugate with chelating agents provided by Dr. Sun's lab for PET imaging and therapeutic peptides provided by Dr. Hsieh's lab for the treatment of aggressive PCa.

BODY

With the ultimate goal to generate a new class of dendrimer-based theranostic agents for aggressive PCa, we have arranged four Specific Aims as indicated in Statement of Work (SOW). Our work in the first year focused on Tasks 1 – 3 to accomplish **Aim 1** and **Aim 2**.

Aim 1: To construct dendrimer conjugates containing specific cell permeation peptides, peptide therapeutic(s) and a bifunctional chelator for PET imaging

Task 1 (Months 1 – 24): Synthesis and Characterization of Dendrimers - Scaffold Library

Please see Dr. Simanek's annual report for the progress of this Task

Task 2 (Months 1 – 12): Synthesis & Characterization of CB-TE2A-based Bifunctional Chelator

As detailed below, my lab has developed an azide-modified form of CB-TE2A, CB-TE2A(^{t}Bu)$_2$-N$_3$, which will conjugate with the alkyne-introduced dendrimers developed in Dr. Simanek's lab.

Aim 2: To select potent compounds with screening systems based on specific mechanism(s) of action

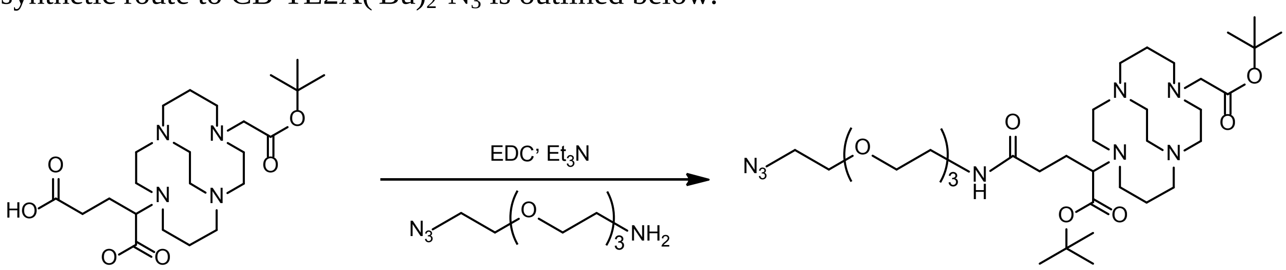
Task 3 (Months 1 – 24). Selection of therapeutic peptides using high throughput assays

Please see Dr. Hsieh's annual report for the progress of this Task

Task 2 (Months 1 – 12): Synthesis & Characterization of CB-TE2A-based Bifunctional Chelator

Fluorine-18 currently plays the dominating role in PET due to the great success of FDG (2-deoxy-2-^{18}F-fluoro-D-glucose). However, its short half-life ($t_{1/2}$ = 109 m) limits its applications to relatively big molecules and requires the radiochemical procedures be performed in the proximity of a cyclotron. Recently, a metal positron emitter, ^{64}Cu has drawn great interest in PET research due to its low positron range, availability, and reasonably long half life ($t_{1/2}$ = 12.7 h), which enables its applications to a variety of molecules, such as peptides, antibodies/fragments,

and even nanoparticles.[1] Given the in vivo PK parameters and biodistribution profiles that we have obtained with the melamine-based dendrimers, ^{64}Cu is particularly suitable for the proposed role of tracking the peptide-dendrimer conjugate delivery in this project. The commonly used chelator, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), can be used as the bifunctional chelator to label the conjugates with ^{64}Cu. Although DOTA-tris (t-Bu ester) is commercially available and can be conveniently used for the construction of DOTA-peptide conjugates, DOTA is not an optimal chelating agent for ^{64}Cu(II), a small metal ion that prefers an octahedral coordination environment.[2] As consequence, relatively high uptake in the liver or non-target organs is observed due to the transchelation of ^{64}Cu from DOTA to other serum proteins. In recent years, my group has focused on the evaluation of optimal chelating agents of Cu(II) for the application of copper radiopharmaceuticals.[2] Among the ligands evaluated, a bicyclo[6.6.2] tetraamine (CB-TE2A: 4,11-bis-(carboxymethyl)-1,4,8,11-tetraazabicyclo[6.6.2] hexadecane) was identified as an ideal chelating agent for the *in vivo* applications of copper radioisotopes. This ligand is capable of adopting conformations having all four nitrogen lone pairs convergent upon a cleft (*in,in* at the bridgehead nitrogens) for complexation of small hard metal ions. The crystal structure of Cu(II)-CB-TE2A reveals that Cu(II) is enveloped in the "clam-shell" of the cross-bridged ligand, where the Jahn-Teller distortions only amount to 2-3% of the Cu-N and Cu-O bond lengths[3], while the axial Cu-O bonds of Cu(II)-TETA or -DOTA are elongated by 6-13% of the equatorial Cu-N bond lengths[4]. Thus, Cu(II) is well-protected from external attacks. *In vivo* evaluation ^{64}Cu-labeled CB-TE2A complexes has demonstrated that they are significantly more resistant to transchelation in liver and more rapidly cleared through non-target organs than currently used TETA/DOTA analogues.[2a,2c,5] As such, we chose CB-TE2A to serve as the ^{64}Cu chelating core for the construction of dendrimer conjugates in this proposal. An azide-modified form of CB-TE2A, CB-TE2A(^{t}Bu)$_2$-N_3, was designed to conjugate with the alkyne-introduced dendrimers via the well-established "click chemistry" procedure. The synthetic route to CB-TE2A(^{t}Bu)$_2$-N_3 is outlined below.



1 2 3

Briefly, to the solution of **1** (0.050 g, 0.095 mmol) in dry acetonitrile (0.5 mL) was added triethyl amine (0.014 g, 0.142 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.027 g, 0.142 mmol). The resulting solution was stirred overnight at room temperature. The solvent was then removed under vacuum to afford the crude product, which was purified by HPLC (Elution time: 22 min) and the resulting fraction lyophilized to give **3** (0.040 g, 0.055 mmol, 58%) as a yellow viscous liquid. ^{1}H NMR (400 MHz, $CDCl_3$): ´ 3.73-3.59 (m, 12H), 3.55 (m, 4H), 3.50-3.34 (m, 8H), 3.33-3.04 (m, 12H), 2.97-2.74 (m, 11H), 1.44 (s, 18H). MS (MALDI) *m/z* calcd for $C_{35}H_{66}N_8O_8$: 726.5; found: 727.8 ($[M + H]^+$). Of note, the two internal carboxylate groups are kept protected with t-butyl so as to avoid the chelation of Cu(II), which might be resulted from the Cu(I) catalyst. To date, we have obtained > 100 mg of Compound **3** by four runs of the reaction.

KEY RESEARCH ACCOMPLISHMENTS

- We have designed and synthesized the proposed bifunctional chelator scaffold system, CB-TE2A(^{t}Bu)$_2$-N$_3$.
- We have extended the chelator scaffold design for noninvasive assessment of prostate specific membrane antigen (PSMA) expression in prostate cancer.
- We have published two peer-reviewed papers: One is a review article that introduces our works-in-progress in the context of recent developments of dendrimer-based theranostic agents; the other is on the use of our proposed bifunctional chelator system to exploit the multivalent effect for the detection of PSMA.

REPORTABLE OUTCOMES

1. Lo S-T, Kumar A, Hsieh J-T, and Sun X: Dendrimer Nanoscaffolds for Potential Theranostics of Prostate Cancer with a Focus on Radiochemistry. *Molecular Pharmaceutics* **2013**, 10:793-812.
2. Hao G, Kumar A, Dobin T, Oz OK, Hsieh, J-T, and Sun X: A Multivalent Approach of Imaging Probe Design to Overcome an Endogenous Anion Binding Competition for Noninvasive Assessment of Prostate Specific Membrane Antigen. *Molecular Pharmaceutics*. **2013**, 10:2975-2985

CONCLUSION

We have designed and synthesized the proposed bifunctional chelator scaffold system, CB-TE2A(^{t}Bu)$_2$-N$_3$ for the further construction of dendrimer-based theranostic agents for aggressive prostate cancer. In the meanwhile, we have extended the design concept to synthesize a bivalent imaging probe for quantitative PET imaging of PSMA expression in prostate cancer.

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